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COMMUNICATION

Remote conformational responses to enantiomeric excess in carboxylate-binding dynamic foldamers

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A crystallographically characterised zinc(II)-capped foldamer can sense the enantiomeric excess of scalemic carboxylate solutions, including those produced by enantioselective organocatalysis, and can relay this input signal along the foldamer body to a remote glycinamide group, which then provides an NMR spectroscopic output.

The determination of the enantiomeric excess (*e.e.*) of scalemic product mixtures produced by enantioselective catalysis is a major research field in organic chemistry and the pharmaceutical sciences.[1,2] Chromatographic separation using chiral solid supports is commonly used to determine *e.e*, but methods that provide a spectroscopic report of the *e.e*. have the benefits of being faster and cheaper.[3]

As part of our work using 3_{10} helical α-aminoisobutyric acid oligomers (Aib foldamers) to sense and transmit signals in organic solvents and phospholipid bilayers,[4] we have become interested in *e.e*. as a signal to be transmitted, particularly as this signal can be generated by an enantioselective catalyst. Previously we showed that capping an Aib octamer with a CuII) bis(2-quinolylmethyl)(2-pyridylmethyl)amine (Cu(BQPA)) group gives a synthetic receptor that responds to the binding of a chiral carboxylate ligand (the input signal) by undergoing a conformational change that propagates over several nanometres either in solution or in a lipid bilayer.[4c] Screening of a series of enantiopure carboxylates showed that the strength of the relayed response depends on the structure of the incoming messenger.[4c]

Simple $Cu(BQPA)^{2+}$ complexes are able to sense the *e.e*. of scalemic mixtures of chiral carboxylic acids.[5] The $Cu(BQPA)^{2+}$ complex adopts a helical conformation and coordination to a chiral carboxylate can favour one conformation over the other. This produces a circular dichroism (CD) signal that, after analysis, indicates the configuration and enantiomeric purity of different acids.[5] Similarly, zinc(II) complexes of tri(2-pyridylmethyl)amine derivatives have been used to the measure the *e.e*. of amino acid or alcohol mixtures.[6]

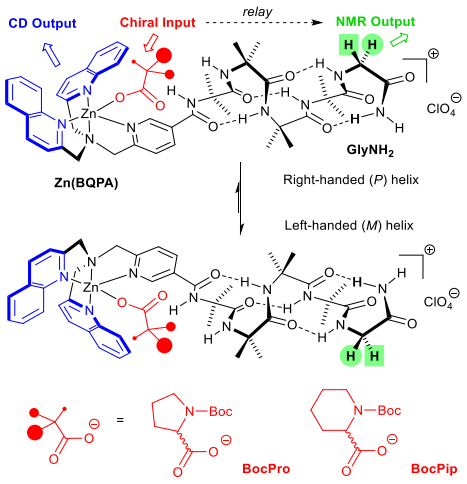

Figure 1: (a) Structure of Zn(**1**)·2ClO_4 after binding a chiral carboxylate (red, BocPro and BocPip shown below), with the equilibrium between screw senses indicated. Quinolyl groups (blue) and diastereotopic protons (green) on the $GlyNH_2$ are highlighted.

Although an N-terminal M(BQPA) on a Aib foldamer should be able to report on the *e.e*. of a ligand mixture, to detect an *e.e*. "signal" that has been relayed along the length of the foldamer requires a reporter with an orthogonal spectroscopic output. Combining a C-terminal glycinamide ($GlyNH_2$) with the N-terminal BQPA would provide a receptor with two orthogonal outputs, namely ^{1}H NMR and CD spectroscopies (Figure 1). The $GlyNH_2$ group is a particularly well-characterised ^{1}H NMR

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reporter group.[4a,7] The 3_{10} helical conformation of the foldamer renders the $GlyNH_2$ methylene protons diastereotopic, giving an AB system where the chemical shift separation ($\Delta\delta$) is proportional to the excess of one screw sense over the other (the helical excess, *h.e.*);[7c,8] an achiral foldamer in fast exchange gives apparently isochronous resonances. Unlike CD, this $GlyNH_2$ reporter~~, however,~~ is insensitive to absolute screw-sense preference (the major conformer is not identified). Replacement of paramagnetic Cu(II) with diamagnetic Zn(II), combined with use of a short tetrameric Aib foldamer that is in fast *M*/*P* helical exchange on the NMR spectroscopy timescale,[8,9] provides the final features found in the receptor Zn(**1**)·$2ClO_4$ (Figure 1). This synthetic receptor should be able to respond to an input signal, the *e.e.* of a scalemic mixture of carboxylates, which could in turn be generated by a catalytic process, and transmit this input signal over the 1 nm length of the foldamer.

BQPA-capped foldamer **1** was synthesised[4c] and complexed to zinc(II) perchlorate to give Zn(**1**)·$2ClO_4$. The X-ray crystal structure was determined for Zn(**1**)·$2ClO_4$ after crystallisation from methanol/diethylether. This structure reveals that, as expected, the Aib tetramer body has folded into a short 3_{10} helix. Some Zn(II) complexes of tris(2-pyridylmethyl)amine have been reported to be *pseudo*-octahedrally coordinated,[10] involving individual or bridging monovalent ligands in some cases,[11] but the structure of Zn(**1**)·$2ClO_4$ shows only a single water molecule (rather than methanol) coordinated to the Zn(II) centre. This water is connected by hydrogen bonds to one of the perchlorate counter ions and then onto the NH of the second Aib residue from the N-terminus. Another network of hydrogen-bonded water molecules also links the bound water molecule to the carbonyl of the BQPA and onto the NH of the third Aib residue. Both indicate how the 3_{10} helix might be influenced by chiral ligands bound to the metal centre. Both screw senses are present in the unit cell, with an *M* propeller-shaped conformation in the BQPA associated with a left-handed (*M*) helical sense in the Aib foldamer ~~body~~chain.

Studies of conformational change in analogous Cu(II)(BQPA)-Aib foldamer complexes had shown that amino acid derivatives bearing *N*-alkoxycarbonyl (e.g. Boc protection) or *N*-acyl (e.g. in a peptide) groups were the most effective.[4c] In particular, BocPro ~~has been shown to produce good chiral induction in the~~is a powerful inducer of conformational chirality in the BQPA derivative of an analogous Cu(**1**) foldamer.

Following previous protocols,[4c] studies of BocPro complexation were performed. The ^{1}H NMR spectrum after the addition of half an equivalent of L-BocPro to Zn(**1**)·$2ClO_4$ in CD_3CN (0.015 M, with 1.2 eq. 2,6-lutidine) shows well-resolved and sharp resonances corresponding to Zn(**1**)·$2ClO_4$ both with and without bound carboxylate (see the ESI, Figure S1), consistent with slow exchange between free and complexed foldamer. The addition of a further 0.5 eq. of L-BocPro led to quantitative formation of the corresponding complex. The resonance from the glycinamide CH_2 shifts upfield by *ca*. 0.25 ppm and splits ($\Delta\delta$ = 181 ppb), consistent with an excess of one screw sense over the other.[12] Increasing the concentration of Boc-L-Pro (up to 4 equiv) gave no further significant spectroscopic changes, consistent with 1:1 binding to Boc-L-Pro, and the $\Delta\delta$ of the $GlyNH_2$ remained constant. The CD spectrum of the Zn(**1**)(L-BocPro) complex showed a positive couplet at 238 nm, and fitting of the data from a CD titration to a 1:1 binding model[13] gave an apparent binding constant of approx. 10^5 M^{-1} (see ESI).

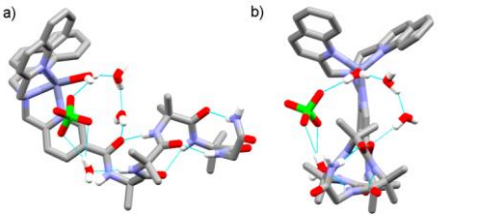

Figure 2. (a,b) X-ray crystal structure of Zn(**1**)·$2ClO_4$. (a) Plan view showing 3_{10}-helix hydrogen bonded to water molecule bound to the zinc ion. (b) View down the axis of the 3_{10} helix. Selected hydrogen bonds shown to illustrate the hydrogen bonded network from the bound water. The perchlorate furthest from the metal centre is not shown for clarity. Methyl, methylene and methine protons are not shown for clarity.

Given that complexed and free Zn(**1**) were in slow exchange with each other, it was not clear whether the NMR output from the $GlyNH_2$ reporter would depend on the *e.e.* of the carboxylate ligand mixture. It seemed possible that anisochronous resonances may result even from addition of *rac*-BocPro~~:~~; each foldamer complexes a single chiral carboxylate of either D or L configuration, which induces splitting of the same magnitude. However, titration of Zn(**1**) with *rac*-BocPro produced a glycinamide CH_2 resonance that had an appearance similar to that of Zn(**1**) alone, *i.e.* split by the adjacent NH but with no apparent anisochronicity. This suggests that the enantiomeric complexes Zn(**1**)(L-BocPro) and Zn(**1**)(D-BocPro) must be exchanging rapidly on the ^{1}H NMR timescale. Subsequent titration of Zn(**1**) (0.015 M in CD_3CN) with scalemic mixtures of BocPro (seven mixtures, 40 to 100 % *e.e.*) confirmed this observation and showed that the chemical shift separation ($\Delta\delta$) between the anisochronous signals from the glycinamide methylene of the foldamer was proportional to the *e.e.* of the BocPro. This change in $\Delta\delta$ with *e.e.* mirrors the expected proportional dependence of ellipticity on *e.e.* (Figure 3), confirming Zn(**1**) is a dual CD and NMR sensor.

To explain this dependence of $\Delta\delta$ on *e.e.*, we propose that in the place of ligand dissociation and re-association, which is demonstrably slow on the NMR timescale (see the ESI, Figure 1), another pathway must exist to facilitate rapid interconversion between coordinated carboxylates (see ESI). We suggest that fast but weak association of an another carboxylate to the zinc(II) centre creates a *pseudo*-octahedral intermediate, which is followed by fast dissociation of one of the complexed carboxylates. This proposed intermediate must be at low concentration, since the ^{1}H NMR spectrum showed no signals that could be ascribed to a hexa-coordinate complex. Lowering the temperature to -40 C (see VT-NMR in ESI) ~~did not~~ provide no more information on the ligand exchange process.

This correlation of *e.e.* and *Δδ* at the $GlyNH_2$ terminus provides the opportunity to create a remote reporter of catalytic activity. Zn(**1**) can report a signal, namely the *e.e*. of a chiral carboxylate mixture, much like sensors that produce a CD output that reports on the *e.e*. of a scalemic mixture. However, Zn(**1**) can relay the signal to a remote site. This *e.e.* signal could be generated by an organocatalytic enantioselective reaction, which would reflect the enantioselectivity of the catalyst.

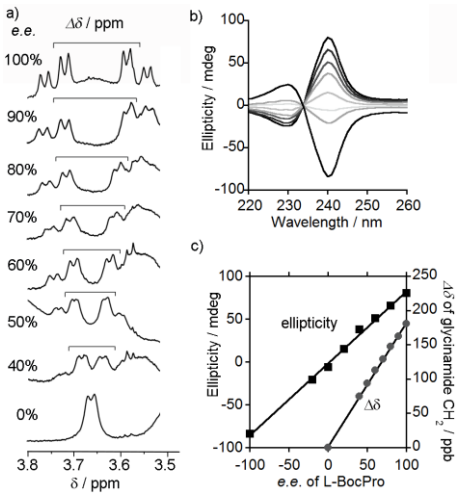

Figure 3: a) Partial ^{1}H NMR spectra (400 MHz, 298 K) showing glycinamide CH_2 region of Zn(**1**)·$2ClO_4$ (15 mM) mixed with scalemic mixtures of D- and L-BocPro (0.075 M with 1.2 eq. of 2,6-lutidine, *e.e*. from 0 to 100%) in MeCN-d_3. b) CD spectra of Zn(**1**)·$2ClO_4$ (0.25 mM) with scalemic mixtures of BocPro (2 equiv.) in MeCN with 2,6-lutidine (2.4 equiv.). c) Change in corrected *Δδ* (black) and ellipicity at the first Cotton effect (λ_{max} = 238 nm, grey) upon addition of scalemic mixtures of BocPro (*e.e.* %) to Zn(**1**)·$2ClO_4$. The *Δδ* values were calculated using published methods (see ESI),[4a] and were unresolved for *e.e.* < 40 %. Linear curve fits shown for ellipticity (R = 0.99825) and *Δδ* (R = 0.99954).

The organocatalytic desymmetrisation of anhydrides was selected as a suitable reaction to demonstrate the recognition and relay of enantioselectivity. Chiral amine **4** catalyses the addition of alcohols to cyclic *meso*-anhydrides to give chiral monoesters with high *e.e*.[14] Anhydride **2a** had been desymmetrised in good *e.e*. (85%, predominately the 1*R*,2*S* isomer) and high yield (90%) by a thiourea analogue of **4**,[15] so desymmetrisation of the same substrate was explored using **4**. Screening indicated that THF gave the best enantioselectivity of those solvents tested (MeOH, 17% *e.e.*; MeCN 64% *e.e.*). Desymmetrisation of **2a** in THF with methanol (10 eq.) and a catalytic amount of **4** (0.1 eq.) afforded monoester **3a** in good yield (98%) and high enantioselectivity (92% *e.e*.) after 24 h. The same conditions were then applied to anhydride **2b**, albeit with a doubling of the reaction time, and resulted in a similarly high *e.e*. and yield (Figure 4).

The resulting scalemic mixture of **3b**, which has a closer structural analogy with BocPro than **3a**, was then added to a solution of Zn(**1**) in acetonitrile and the resulting mixture was analysed by CD and NMR spectroscopy. The addition of aliquots of **3b** (1*R*, 2*S*) to Zn(**1**) (0.025 mM) gave a positive exciton-coupled circular dichroism (ECCD) signal[5] at 238 nm in the CD spectra due to an exciton couplet between the quinoline chromophores. The sign of the change suggests **3b** (1*R*, 2*S*), much like (*S*) amino acids, causes a left-handed (*M*) propeller twist in the BQPA.[5] A plot of the intensity of the signal with respect to **3c** concentration reached a maximum of +19 mdeg after 0.6 equiv., perhaps due to the formation of bridged 2:1 foldamer:carboxylate complexes, before declining.

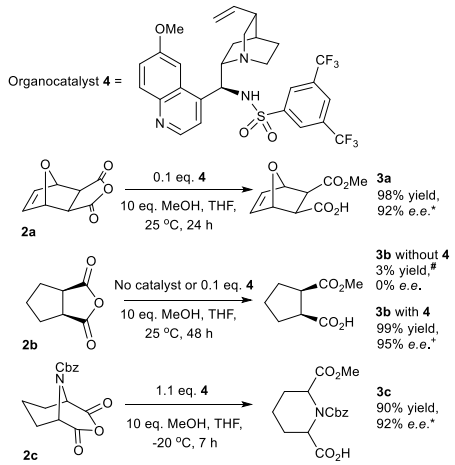

Figure 4: Organocatalytic desymmetrisation of cyclic *meso*-anhydride**s** by **4** to give scalemic mixtures of chiral carboxylic acids. Major isomer shown. Stereochemistry confirmed (where indicated) by comparison of measured $[\alpha]_D$ values to reported values. # Not separated from anhydride. Enantiomeric excesses (*e.e.*) determined by HPLC on chiral stationary phase (*) or ^{1}H NMR spectroscopy after derivatization with (*R*)-naphthylethylamine (+).

For ^{1}H NMR spectroscopy studies, the monoester **3b** was separated from **4** by washing with 1M HCl: although **4** did not itself induce splitting in the glycinamide CH_2 resonance, it did obscure key resonances. The unresolved monoester mixture was then dissolved in CD_3CN (75 mM, with 1.2 eq. 2,6-lutidine). Monoester **3b** was added into Zn(**1**) in CD_3CN (15 mM) to give a 1:2 foldamer:carboxylate ratio and the corresponding ^{1}H NMR spectrum recorded. A low *Δδ* value (below 40 ppb) was observed (Table 1). Similarly, **3a** and the cyclohexane monoester analogue of **3b** (**3d**, >99 % *e.e*.) both also gave low *Δδ* values (44 ppb and 39 ppb, see the ESI). The racemic mixture from the uncatalysed reaction of **3b** gave a *Δδ* of 0 ppb, which shows that, as expected, the C-terminal $GlyNH_2$ reports the *e.e.* of the scalemic mixture, rather than an individual bound signal molecule.

These Δδ values are much lower than those for L-BocAla, L-BocPro and L-BocPip (*Δδ* = 127, 181 and 231 ppb respectively). For these carboxylates and **3b**, the magnitude of *Δδ* does not correlate well with the strength of the CD signal (Table 1), showing that the ability to induce conformational change at

the BQPA does not necessarily translate into good signal transmission down the helix. This suggests that screw-sense preference originates directly from the ligand, perhaps through hydrogen bonding into the Aib backbone.[4c]

Based upon this hypothesis, a new substrate with a hydrogen bond acceptor adjacent to the amine, **2c**, was designed. Anhydride **2c** is structurally similar to BocPip (Figure 1), which induces a high *h.e.* in Zn(**1**) (Table 1). Anhydride **2c** was synthesised from pyridine-2,6-dicarboxylic acid (see the ESI) and subjected to methanolysis catalysed by **4**. Although this substrate was less effectively desymmetrised under standard conditions (25 °C, 1-2 days), a significant increase in the loading of **4** (from 0.1 to 1.1 eq.) and a decrease in reaction temperature (to −20 °C) provides monoester **3c** in good yield and 92% *e.e.*[16] As hoped, **3c** displayed better control over helical sense, although this control ($\Delta\delta$ = 90 ppb) was still less than that induced by BocPip ($\Delta\delta$ = 231 ppb).[17]

Table 1: Table of carboxylate *e.e.* with corresponding ellipticity induced at the N-terminal BQPA (ΔA, ECCD intensity at the first Cotton effect λ_{max} = 238 nm) and anisochronicity ($\Delta\delta$) induced in the C-terminal $GlyNH_2$ of Zn(**1**).$2ClO_4$.

Input	*e.e.*	ΔA^a / mdeg	$\Delta\delta^{a,b}$ / ppb
L-BocAla	~100%	+43	127
L-BocPro	~100%	+80	181
L-BocPip	~100%	+53	231
3b	0%	0	0
3b	95%[1,+]	+13	29

[a] CD and $\Delta\delta$ values were determined after addition of 2 eq. of the carboxylate. [b] Chemical shift separations of the glycinamide protons determined at 25 °C in CD_3CN. Uncertainties in ΔA are approx. ±10%.

In summary, a novel Zn(BQPA)-capped dynamic Aib foldamer has been synthesised, structurally characterised and shown to act as a CD and remote NMR sensor of *e.e.* in scalemic mixtures of carboxylates. This foldamer transmits an input signal, the *e.e.* of each scalemic mixture, along the length of foldamer to the reporter group. This type of signal transduction differs from those previously reported for Aib foldamers, in that the foldamer senses and responds to a system (an ensemble of signalling molecules) rather than the presence of a specific signalling molecule. By using this signalling relay to sense the outcome from enantioselective organocatalysis, proof-of-concept for signal conversion and amplification has been demonstrated; an unreported chiral signal (catalyst **4**) has been converted into a detectable signal that is amplified and transmitted down the foldamer length.

Conflicts of interest

There are no conflicts to declare.

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17 Residual catalyst in the **3c** mixture interfered with the measurement of the BQPA ECCD signal from Zn(**1**)·$2ClO_4$.

Commented [JC1]: It's different in concept, but we did use a mixture of signalling molecules in JACS 2015 6680, using pH to select between them.